well as synthetically evolved gain-of-function variants of melittin, which have recently been developed by William Wimley at Tulane University.
Using spectroscopic techniques we show that gain-of-function variants are able to leak larger dyes from unilamellar vesicles than melittin. Like melittin gain-of-function variants result in virtually complete leakage of the dye from vesicles. However, the concentration of peptide required to achieve leakage is significantly lower for gain-of-function variants than for melittin. This suggests that these peptides form larger pores than melittin and that these pores are much more stable, remaining functional over the lifetime of the leakage experiment. Simulations of both melittin and gain-of-function variants reveal a wealth of atomic detail information about transient processes such as peptide absorption, folding, and oligomeric assembly, as well as the equilibrium structural ensemble and stability, which were verified using circular dichroism, fluorescence, and electrochemical impedance spectroscopy.

2513-Plat
The Curvature Induction of Surface-Bound Antimicrobial Peptides Piscidin 1 and Piscidin 3 Varies with Lipid Chain Length
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The initial steps of membrane disruption by antimicrobial peptides (AMPs) involve binding to bacterial membranes in a surface-bound (S) orientation. To evaluate the effects of lipid composition on the S state, molecular dynamics simulations of the AMPs piscidin 1 (p1) and piscidin 3 (p3) were carried out in 4 different bilayers: 3:1 DMPC/DMPG, 3:1 POPC/POPG, 1:1 POPE/POPG, and 4:1 POPC/cholesterol. In all cases, the addition of 1:40 piscidin caused thinning of the bilayer, though thinning was least for DMPC/DMPG. The peptides also insert most deeply into DMPC/DMPG, spanning the region from the bilayer midplane to the head groups, and thereby only mildly disrupting the acyl chains. In contrast, the peptides insert less deeply in the palmitoyl-oleoyl containing membranes, do not reach the midplane, and substantially disrupt the chains; i.e., the neighboring acyl chains bend under the peptide, forming a basket-like conformation. Curvature free energy derivatives calculated from the simulation pressure profiles reveal that the peptides generate positive curvature in membranes with palmitoyl and oleoyl chains but negative curvature in those with myristoyl chains. Curvature inductions predicted with a continuum elastic model follow the same trends, though the effect is weaker and a small negative curvature induction is obtained in POPC/POPG. These results do not directly speak to the relative stability of the inserted (I) states or ease of pore formation, which requires the free energy pathway between the S and I states. Nevertheless, they do highlight the importance of lipid composition and acyl chain packing.

2514-Plat
A General Mechanism for Off-Target Effects: Studies with Amiodarone and other Antiarrhythmics
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Atrial fibrillation (AF) is the most prevalent type of arrhythmia and is associated with significant mortality. Amiodarone, owing to its efficacy and minimal proarrhythmic effects, is the most commonly prescribed antiarrhythmic agent. Though amiodarone is considered a class III, repolarization-prolonging, antiarrhythmic that inhibits potassium channels involved in restoring the membrane excitable state, it also alters the function of many other structurally and functionally diverse membrane proteins. This concomitant regulation of multiple membrane proteins by amiodarone results in complex therapeutic and toxicity profiles. Other antiarrhythmics, such as dronedarone, have similar multiple membrane protein targets. Though such a multipronged mechanism for treating AF appears to be desired, it is not clear how amiodarone, and other antiarrhythmics exert their therapeutic actions and regulate a diverse range of membrane proteins at similar concentrations. Chatelane et al. (1989) found that amiodarone and propranolol alter lipid bilayer properties, and that amiodarone does so at therapeutic concentrations. We therefore took advantage of the gramicidin (gA) channels' sensitivity to changes in the lipid bilayer properties to determine whether the commonly used antiarrhythmics amiodarone, dronedarone, propranolol and pindolol perturb the lipid bilayer properties and at which concentrations. Using a gA-based fluorescence assay and single-channel electrophysiology to explore the antiarrhythmics' effects on the lipid bilayer, we found that amiodarone and dronedarone are potent bilayer modifiers, propranolol has intermediate activity, and pindolol is the least potent. Moreover, amiodarone and propranolol increase bilayer elasticity. Because amiodarone and dronedarone alter the lipid bilayer at their therapeutic concentrations, where they act on multiple membrane proteins, our results suggest that their multi-target effects may involve a lipid bilayer-mediated mechanism. This underscores the need to further explore the role of bilayer-mediated mechanism in therapeutic as well as toxic effects of antiarrhythmics agents.

Symposium: Advances in Electron Microscopy

2515-Symp
Cryo-EM of DNA Repair Protein Complexes
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The most recent technical advancement of cryo-electron microscopy is revolutionizing structural biology. Using the new generation of transmission electron microscope and direct electron counting device, we can now solve the structure of important DNA repair complexes with high efficiency and accuracy so to observe new structural features unseen.

2516-Symp
Structural Maturation of Hepatitis B Core Protein Capsids
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Hepatitis B virus consists of a capsid formed by icosahedrally arranged Hepatitis B core protein (HBC) and an envelope with three different types of membrane integrated surface proteins (HBS). During viral maturation, inside the capsid the RNA-pregenome is reversely transcribed into a partly double stranded DNA. The reverse transcription is concomitant with the dephosphorylation of HBC. Only after the reverse transcription is completed the viral capsid is enveloped. Interestingly a single, naturally occurring point mutation I/F97L in HBC causes premature envelopment of the capsid.
We have used electron cryo microscopy and image processing to investigate the structure of phosphorylated and unphosphorylated HBC cores as well as of the premature envelopment mutant to understand the structural mechanisms of capsid maturation.

2517-Symp
Single Particle Cryo-EM of Calcium Release Channels
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Ca^{2+} Release Channels (CRCs) are intracellular ligand-gated Ca^{2+} channels that are responsible for an increase of cytosolic Ca^{2+} levels in response to diverse stimuli. Two closely related families, ryanodine receptors (RyRs) and inositol 1,4,5-trisphosphate receptors (IP_3Rs), constitute this class of the tetrameric ion channels localized in SR/ER membranes of all eukaryotic cells. Despite long-established efforts of multiple groups, structural analysis of CRCs have proven difficult due to their inherent dynamic nature and their enormous size (1.3 MDa for IP_3Rs and 2.3 MDa for RyRs), making X-ray or NMR techniques poorly suited for structural studies of these membrane proteins. The best current structures of complete CRC, determined by single particle electron cryo-EM at intermediate resolutions of 10-15 Å, reveal some secondary structure elements in both the cytoplasmic and transmembrane regions of channels. Recent advances in cryo-EM field, including the use of the direct electron detection cameras and improved image-processing software, have, in some favorable cases, allowed for the determination of near-atomic resolution structures of integral membrane proteins. Our strategies and recent progress toward high-resolution structure determination of the entire CRC will be discussed in the context of breakthrough developments in single-particle cryo-EM.
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2518-Symp
Single Particle CryoEM of Integral Membrane Proteins
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As a versatile tool in structural biology, single particle electron cryo-microscopy (cryo-EM) has achieved milestones of determining near atomic resolution three-dimensional (3D) reconstructions of non-enveloped viruses with icosahedral symmetry. Recent technological breakthroughs in single particle cryo-EM, particularly the development of novel dose-fractionated image